Electromagnetic tracking of handheld high-resolution endomicroscopy probes to assist with real-time video mosaicking

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ABSTRACT

Optical fiber bundle based endomicroscopy is a low-cost optical biopsy technique for *in vivo* cellular level imaging. A limitation of such an imaging system, however, is its small field-of-view (FOV), typically less than 1 mm^2. With such a small FOV it is difficult to associate individual image frames with the larger scale anatomical structure. Video-sequence mosaicking algorithms have been proposed as a solution for increasing the image FOV while maintaining cellular-level resolution by stitching together the endomicroscopy images. Although extensive research has focused on image processing and mosaicking algorithms, there has been limited work on localization of the probe to assist with building high quality mosaics over large areas of tissue.

In this paper, we propose the use of electromagnetic (EM) navigation to assist with large-area mosaicking of hand-held high-resolution endomicroscopy probes. A six degree-of-freedom EM sensor is used to track in real-time the position and orientation of the tip of the imaging probe during free-hand scanning. We present a proof-of-principle system for EM-video data co-calibration and registration and then describe a two-step image registration algorithm that assists mosaic reconstruction. Preliminary experimental investigations are carried out on phantoms and *ex vivo* porcine tissue for free-hand scanning. The results demonstrate that the proposed methodology significantly improves the quality and accuracy of reconstructed mosaics compared to reconstructions based only on conventional pair-wise image registration. In principle, this approach can be applied to other optical biopsy techniques such as confocal endomicroscopy and endocytoscopy.

Keywords: Endomicroscopy, optical fiber bundle, mosaicking, fluorescence, electromagnetic tracking

1. INTRODUCTION

Malignancies of the gastro-intestinal (GI) tract, such as oesophageal cancer and colon cancer, have the fastest rising incidences in the US and Europe, and represent one of the leading causes of cancer-related deaths worldwide [1]. The primary reason for the low survival rate in GI cancer is largely attributed to its advanced stage at diagnosis [2]. More than 80% of cancers originate from the epithelial tissues and hence endoscopic biopsy procedures have been the primary clinical platform to detect regions of possible neoplasia. In a standard procedure, a flexible endoscope is inserted through the mouth of the patient and guided through the oesophagus to the tissue under examination using endoscopic guidance. Biopsies are then acquired from suspicious tissue regions by excising small samples of the tissue in approximately 1-2 cm intervals and then imaging it under a light microscope for histopathology analysis. This point based invasive approach may lead to significant sampling errors, trauma and tissue damage. Moreover, cyto-histopathology analysis requires special preparation of the dissected tissue (fixation, slicing and staining) prior to its examination under a microscope thus making the final diagnosis time-consuming, as well as being dependent on the skill and experience of trained specialists.

To overcome these limitations, in recent years various optical techniques for microscopic imaging of cellular structures in a minimally invasive way have received attention. These techniques, based on the principle of 'optical biopsy', offer potential advantages over routine biopsies, including non-invasiveness, *in vivo* real-time imaging, fewer sampling errors, and reduced tissue damage, and are slowly approaching the resolution of conventional histopathology imaging [3–5]. High resolution fiber bundle endomicroscopy is one such recently developed optical biopsy technique that translates conventional microscopy into an *in vivo* clinical imaging modality. The fiber bundle probe, integrating wide-field optics [6–8] or confocal optics [9] in the distal end of a flexible endoscope, facilitates fluorescence imaging of biological tissue at

confined sites within the body with a flexible fiber bundle. Further, the probe can be integrated into standard video endoscopes and hence has emerged as a popular tool for minimally invasive endoscopic procedures, finding many applications in endoscopic imaging of the GI tract in preclinical and clinical studies.

However, image acquisition using fiber bundle based endomicroscopy systems offers several challenges. A major limitation being that the field of view (FOV), limited by the size of the fiber bundle, is typically less than 1 mm^2. Such a low FOV reduces the amount of morphological features visualized, particularly while imaging long segments of oesophagus. Researches in this field have extensively used image mosaicking algorithms (stitching of adjacent images as the probe moves) to increase the FOV in the direction of the probe movement while still maintaining microscopic-level resolution. For endomicroscopy procedures, the mosaicking algorithms involve stitching together small FOV images obtained by scanning the probe over the tissue surface and generate a wide FOV image [10–12]. Classical mosaic reconstruction algorithms, however, rely solely on the image data to estimate the probe motion between adjacent images. The accuracy of the mosaic reconstruction is thus dependent on the precision and consistency with which the probe is scanned over the tissue. Large area mosaicking is therefore challenging, given the obvious difficulties in achieving these slow, precise and controlled hand-held movements. Furthermore, image based mosaicking algorithms do not take into consideration tissue geometry and the position and orientation of the imaging probe during reconstruction of the scan trajectory and hence cannot provide information about the true 3D anatomical structure of the tissue under examination. Despite significant research on image processing and mosaicking algorithms, there has been limited work on localization of the probe to assist with building high quality mosaics for freehand scanning applications. Recently a few approaches on the use of robotic actuation of endomicroscopy probes [13–15] have been proposed to minimize probe motion artefacts during scanning over large tissue areas as well as to use visual servoing to compensate for robotic and image motion due to tissue sweeping[12]. However these methodologies are developed particularly for robotically actuated probes and cannot be generalized for more common freehand scanning or steering applications.

To address the aforementioned concerns, we propose a new framework based on the fusion of anatomical images from wide-field fluorescence endomicroscopy and the corresponding probe pose from an electromagnetic (EM) sensor to reconstruct mosaics with true 3D scan trajectory. EM tracking facilitates the localization of lesion sites by accurately tracking the 3D position and orientation of instruments. It has found extensive applications in flexible bronchoscopy [16] and endovascular surgery [17], along with recent applications of tracker-assisted needle optical coherence tomography (OCT) probe for lung imaging [18,19]. In this paper we present a proof-of-principle system for EM-endomicroscope assembly and propose a co-calibration, registration and data-fusion algorithm to integrate the tracking data into the mosaicking software. Preliminary experimental investigations are carried out for freehand scanning on rigid paper phantoms and *ex vivo* porcine colon tissue. The details of the sensor integration, software framework, system performance analysis and improvements made possible by these investigations, compared to reconstructions based only on conventional pair-wise image registration, are presented in the following sections.

2. METHODOLOGY

2.1 Hardware configuration

A schematic of the wide-field endomicroscopy system is shown in Figure 1. The system follows an experimental design reported in earlier literature [6–8]. A royal blue LED (Thorlabs M455L3) with a 450 nm low-pass excitation filter is used as the source of illumination. A dichroic filter (Thorlabs DMLP505R) is used to direct the excitation light through a microscope objective (Edmund Optics #67-706; 0.15 NA) into the proximal end of the 'probe' which consists of a fiber-bundle and micro-lens assembly (Cellvizio Gastroflex UHD, Mauna Kea Technologies). The probe is 3 meters in length with maximum outer diameter of 2.6 mm, a core spacing of 3 μm, a lateral resolution of approximately 2 μm and a usable circular field-of-view of 240 μm. The excitation illumination is transferred coherently from the proximal to the distal end. The distal end is placed in direct contact with the tissue/specimen and the fluorescent emission is collected by the bundle and focused onto a monochrome CCD camera (Thorlabs DCU223C) via the dichroic filter, an emission filter (Thorlabs FEL0500) and a tube lens (Thorlabs AC254-050-A), with a net magnification M = 2.5. The magnification factor ensures that the image of the probe is confined to the physical area of the CCD array. Images are acquired at 15 frames per second (fps) framerate.

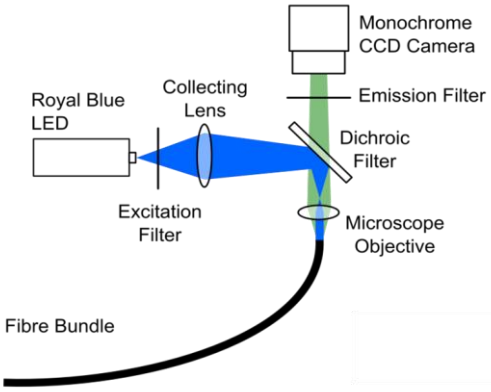

Figure 1. Optical layout of high resolution wide-field fluorescence endomicroscopy system

The corresponding 3D probe pose information is recorded from the EM sensor which is rigidly fixed to the distal end of the endomicroscopy probe in order to track its tip position at all times. An EM sensor with 6 degrees of freedom (DOFs) is used with the Aurora EM Tracking System (NDI Corporation, Waterloo, Canada) to collect the probe position and orientation information. In this proof-of-concept study a non-metallic hand-held probe holder consisting of the imaging probe and an EM sensor is designed and used for the preliminary experimentation. In future this assembly can be further miniaturized and re-designed to pass through the working channel of conventional video endoscope.

2.2 Image acquisition and processing

The image acquisition process is controlled by a software package developed in Labview (NI) by our laboratory. A single image frame with a resolution of 600x600 pixels is captured using the wide-field endomicroscopy imaging system. For these recorded raw image frames, it is necessary to remove the honeycomb-like fiber bundle pixelation artefacts that arise due to presence of non-imaging spaces between adjacent fiber cores. To remove this pattern, the raw images are convolved with a 2D Gaussian kernel with a standard deviation (sigma) of 1.4 pixels (equivalent to 1.2 µm on the face of the fiber bundle). Next, an image intensity correction is implemented to compensate for variations due to non-uniform illumination intensity and coupling efficiency of individual fibers in the fiber bundle by calculating a 2D intensity correction map and multiplying it with the previously reconstructed image. Finally a circular window is applied to remove the edges of the fiber bundle. An image frame acquired by the endomicroscopy system before and after pre-processing is shown in Figure 2. The image acquisition and processing is carried out at 15 fps for live display to the user or for recording to an AVI video.

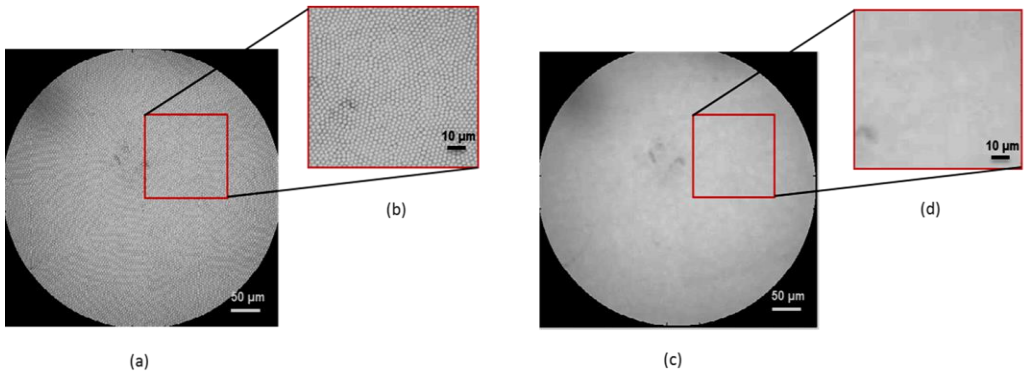

Figure 2. Removal of fiber pixelation artefacts a) Raw endomicroscopy image of background illumination, b) zoomed image showing honeycomb pattern of cores, c) processed image using Gaussian filter, d) zoomed processed image showing that fiber-pixelation is no longer visible.

2.3 EM tracker performance

Even-though the reported absolute accuracy of the EM sensor is 1.4 mm RMS, for image mosaicking applications it is the relative accuracy between current pose and start position that is of critical importance. We assess the relative position accuracy of the EM sensor and compare it against the gold standard translation stage data by adopting an experimental evaluation similar to that documented by Lau *et. al.* [18]. The EM sensor was mounted on the translational stage using a non-metallic rod such that the z-axis of the EM sensor was directed towards the z-axis of the Aurora field generator. The sensor was then translated using a 2D translation stage and tracker data was recorded for translations in the lateral and axial directions of the field generator. Experimental results were evaluated for two conditions of using: i) only the EM sensor and ii) EM sensor integrated with the endomicroscope probe. For distances up to 20 cm from the field generator, the relative accuracy of raw tracker data was measured to be 40 μm ± 10 μm along the y-direction (lateral scanning) and 80 μm ± 10 μm along the z-direction (axial scanning) in the field-generator co-ordinate space. The presence of metal on the tip of the endomicroscope probe was not found to influence the EM tracker accuracy results.

2.4 Calibration and Registration methodology

Prior to EM-endomicroscope data registration, a method for calibration of EM-endomicroscope data transformation is implemented. In future, the two probes could be permanently combined into a single unit in future, meaning that this would be a one-time calibration. The calibration procedure has two parts: *Temporal calibration* to find the accurate synchronization of the time at which the endomicroscope image is captured with the time at which position information is read from the EM sensor, and *Spatial calibration* to find the accurate geometric transformation between the endomicroscope image co-ordinate system and EM sensor co-ordinate system.

For our work, both the EM sensor and image data are time-stamped and we use these time-stamp measurements for temporal calibration. We assume that the time stamps represent the exact time of measurement and perform temporal calibration via 1D linear interpolation.

We implement a motion-based method of the form *'AX=XB'* [20,21] which is commonly seen in hand-eye calibration literature to calibrate the spatial transformation between endomicroscope image and EM sensor data. This data is compared with the translational stage data, which is the gold standard for our validation. The geometric transformation is expressed as:

$$ {}^{F}T_{I} = {}^{F}T_{E}\ {}^{E}T_{I} \tag{1} $$

$$ {}^{F}P_{I} = {}^{F}T_{I}\ {}^{I}P_{C} + T_{offset} \tag{2} $$

where, '${}^{F}T_{I}$' is the desired co-ordinate transformation between the endomicroscope image co-ordinates and the Aurora field generator coordinate system, '${}^{F}T_{E}$' is the co-ordinate transformation between the EM sensor and the Aurora coordinate system, '${}^{E}T_{I}$' is the co-ordinate transformation between the endomicroscope image co-ordinate system and EM sensor, ${}^{F}P_{I}$ corresponds to the 3D image co-ordinates expressed in millimeters in the 3D Aurora co-ordinate system and ${}^{I}P_{C}$ corresponds to the 2D pixels co-ordinates expressed in millimeters in the 3D image co-ordinate system.

Since the EM sensor is rigidly attached to the endomicroscope probe, it is assumed that the endomicroscope probe and the EM sensor share the same orientation due to the small distance between them as well as due to high rigidity of the two probe tips. Hence there is a fixed geometric relation given by the translational component 'T_{offset}' between the two motions.

The transformation '${}^{F}T_{E}$' can be calculated from the position and orientation information obtained from the data recorded using the Aurora EM system. The 3D rotation matrix used for this transformation is given by

$$R=\begin{bmatrix} \cos\gamma\cos\alpha & \cos\alpha\sin\gamma & -\sin\alpha & 0 \\ \cos\gamma\sin\alpha\sin\beta-\sin\gamma\cos\beta & \cos\gamma\cos\beta-\sin\alpha\sin\beta\sin\gamma & \cos\alpha\sin\beta & 0 \\ \sin\gamma\sin\beta-\cos\gamma\cos\beta\sin\alpha & \sin\gamma\cos\beta\sin\alpha-\cos\gamma\sin\beta & \cos\alpha\cos\beta & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \quad (3)$$

where α, β and γ correspond to the roll, pitch and yaw angles respectively of the EM sensor measured in the Aurora field generator co-ordinate space.

2.5 Mosaic reconstruction by EM-endomicroscope data fusion

In order to reconstruct mosaics with benefits both from the image data and EM data, a 2-step EM-endomicroscope data fusion algorithm is implemented. A coarse image alignment is first performed based on the EM sensor data for all the image frames. Following this, a fine-tuning by normalized cross-correlation (NCC) is performed only on a subset of image frames. The criteria for selection of frames are based on a correlation coefficient thresholding algorithm.

A fast NCC algorithm using Fourier transforms is used for pair-wise image registration based on template matching to evaluate, in one pass, the correlation coefficient between consecutive image frames ' I_k ' and ' I_{k+1} '. A 2D correlation map is generated using:

$$C_{NCC}(u,v)=\frac{\sum\left(I_k(x,y)-\bar{I}_k\right)\left(I_{k+1}(x-u,y-v)-\bar{I}_{k+1}\right)}{\sum\left(I_k(x,y)-\bar{I}_k\right)^2+\sum\left(I_{k+1}(x-u,y-v)-\bar{I}_{k+1}\right)^2} \quad (4)$$

where ' $\overline{I_n}$ ' is the average pixel value of the image ' I_n ' x, y are pixel co-ordinates and u, v is the translational shift. The translational shift is obtained from maximum correlation peak using:

$$\tilde{C}(I_k,I_{k+1})=\arg\max\left(C_{NCC}(u,v)\right) \quad (5)$$

For our experiments the NCC threshold is set at a value of 0.9. Following the registration, the endomicroscope images are placed appropriately on the reconstruction canvas using co-ordinate transformations to reconstruct a large FOV output mosaic in 2D or 3D space.

3. RESULTS

Figure 3 shows representative imaging results from a rigid paper phantom with a circular trajectory printed on it and from topically stained *ex vivo* porcine colon tissue samples with 0.02% proflavine. All experiments are carried out in controlled environments to minimize the effect of distortions due to metal structures on EM measurement accuracy. The image frames were extracted from videos, acquired at 15 fps, during freehand scanning of the hand-held endomicroscope probe-EM sensor assembly on the phantom (or tissue) surface. The acquired raw images were pre-processed to remove the fiber-pixelation artifacts using an existing GUI software platform developed in LABVIEW® in our laboratory.

Using the pose information from the EM sensor and transformation matrices obtained during the calibration process, the 2D image points on the processed endomicroscope images were transformed to 3D points in the Aurora co-ordinate system. The corresponding mosaics are reconstructed following a lateral scan on the specimen surface using (i) conventional NCC based image registration, and (ii) the proposed EM-endomicroscope data fusion method with correlation coefficient thresholding. For these experiments, the lateral probe displacement is much larger than the lateral resolution of the imaging system. The results show that with the proposed methodology of scanning over a closed-loop circle, the experimentally evaluated scan trajectory follows the reference trajectory more accurately over the full measurement range. For lateral scanning on ex-vivo colon tissue, the probe motion artefacts and soft tissue deformations often result in inconsistencies in the quality of the captured images and thus affect the accuracy of mosaics reconstructed using image based registration. The results show that the additional information from the EM sensor facilitates more accurate positioning of these inconsistent images and leads to mosaic reconstruction with improved quality and accuracy as compared to conventional NCC based image registration algorithm.

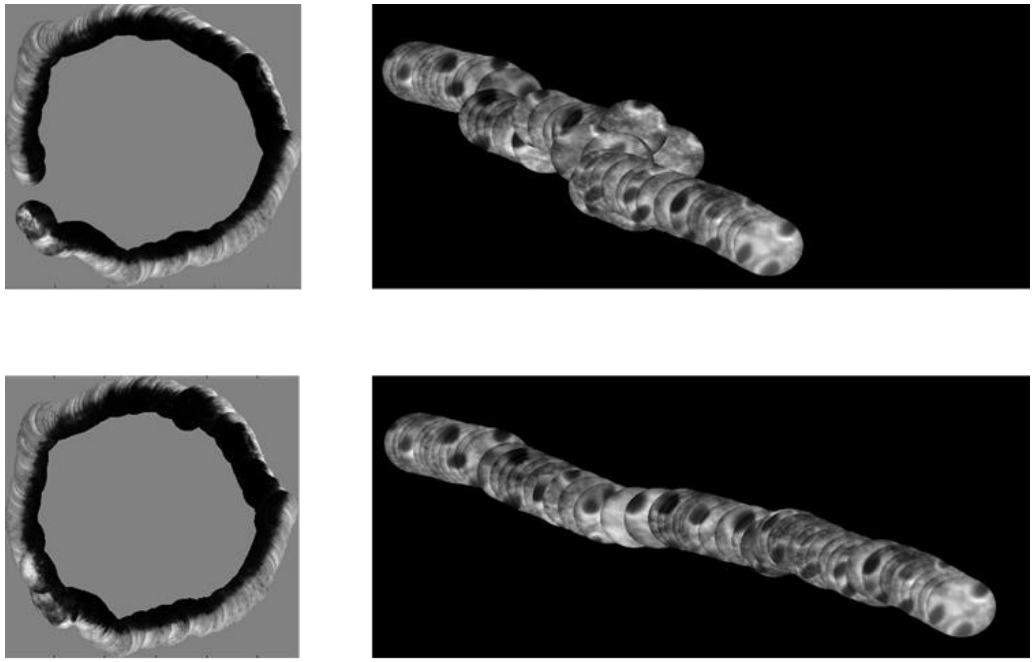

Figure 3. Examples of image mosaics of endomicroscopy images reconstructed using NCC based image registration (Top Row) and the proposed EM-endomicroscopy fusion method (Bottom Row). Showing (i) close-loop trajectory tracing on a printed pattern of a circle and (ii) lateral scanning on 0.02% proflavine stained ex vivo porcine colon tissue

4. DISCUSSION

In this paper, we propose an EM-assisted fiber bundle endomicroscopy system for endomicroscope probe localization and large area mosaic reconstruction during freehand scanning. From the experiments it is observed that during freehand scanning the acquired endomicroscope video sequence often consists of image frames with inconsistencies in image features and quality due to various factors such as soft-tissue deformation, improper probe-tissue contact and variations in scanning speed and orientation of the probe. Simple image registration algorithms do not account for these changes during mosaicking, and thus may result in mosaic reconstruction with geometric distortions and sub-optimal accuracy, particularly while scanning over large areas. However it does provide good small-scale accuracy when consecutive image frames have sufficient overlap (> 75%) and identifiable image features.

By combining the NCC based image registration with the EM tracker measurements using the proposed data fusion algorithm, we are able to use the strengths of each of these techniques to improve the overall mosaic reconstruction accuracy. For this study, preliminary experimental investigations were carried out on phantoms and *ex vivo* porcine tissue for free-hand scanning using a wide-field endomicroscope imaging system. The results demonstrate that the proposed methodology significantly improves the quality and accuracy of reconstructed mosaics compared to reconstructions based only on conventional pair-wise image registration. For this proof of principle study we are directly using the raw EM tracker data but for future work it would be possible to improve the spatial accuracy of the EM sensor further by correcting for ferromagnetic distortions using static and dynamic error compensation methods such as moving-average based filtering, interpolation or polynomial fitting techniques.

A key advantage of this method lies in its potential for miniaturization. Due to the small size of both the EM sensor and the endomicroscope probe, an integrated probe could be deployed through the working channel of a standard video endoscope. Hence it would be feasible to use this as a visualization tool during endoscopic procedures. Further experiments will be conducted to re-design the probe assembly by optimizing the location of attachment of the EM

sensor on the endomicroscope probe and to carry out *in vivo* imaging by deploying it through the endoscope channel. Another advantage is that the proposed method in principle can be applied to other fiber bundle optical biopsy techniques such as confocal endomicroscopy, endocytoscopy or dual-mode fiber bundle endomicroscopy by modifying the proximal optics of the experimental system.

An alternative approach to minimize the influence of artifacts due to probe motion other than by using EM navigation is to use a high frame rate imaging system that ensures sufficient degree of overlap between consecutive image frames. Our laboratory has recently developed a high-speed line-scanning confocal endomicroscope that can achieve frame rates upto 120 fps, an order of magnitude improvement over commercially available microconfocal systems. Initial feasibility work on topically stained ex vivo gastrointestinal tissues and in vivo rectal mucosal study in a porcine model demonstrated that it was feasible to obtain high quality real time image mosaics with wider field of views while still maintaining sufficient resolution and signal-to-noise ratio and the performance of mosaicking was improved as compared to low frame rate systems [22]. Further experiments involving a study of wide range of tissue specimens and topical staining agents for *ex vivo* and *in vivo* imaging applications using different fiber bundle endomicroscopy systems with high and low image acquisition rate will be investigated to realize the full potential of the proposed methodology.

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